

## AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions of claims in the application. Please replace claim 6 with the following amended claim, cancel claims 1-3, 5, 10, and 22-34 without prejudice, and add new claims 35-43.

### Listing of Claims:

1-5 (Cancelled)

6. (Currently Amended) A method for detecting an increased risk of developing a neural tube defect, Down's Syndrome, or cardiovascular disease in a mammalian embryo or fetus, said method comprising detecting the presence of a polymorphic methionine synthase reductase (MTRR) in a test subject, wherein said test subject is a future parent of said embryo or said fetus, and wherein detection of a homozygous MTRR polymorphism in said future parent, said embryo, or said fetus, or detection of either a homozygous or heterozygous MTRR polymorphism in both future ~~parents~~ parents, indicates an increased risk of developing said neural tube defect, Down's Syndrome, or cardiovascular disease in said embryo or said fetus, wherein said polymorphism comprises

(a) a G instead of an A at position 66 relative to the first nucleotide of the start codon of MTRR,

(b) a G instead of an A at position 110 relative to the first nucleotide of the start codon of MTRR,

(c) a deletion of 4 nucleotides starting from position 1675 (nucleotides 1675-1678) relative to the first nucleotide of the start codon of MTRR, or

(d) a deletion of 3 nucleotides starting from nucleotide 1726 (nucleotides 1726-1728) relative to the first nucleotide of the start codon of MTRR.

7. (Original) The method of claim 6, wherein said polymorphic MTRR is detected by analyzing nucleic acid from said test subject.

8. (Original) The method of claim 7, wherein said nucleic acid is genomic DNA.
9. (Original) The method of claim 7, wherein said nucleic acid is cDNA.
10. (Cancelled)
11. (Original) The method of claim 7, said method further comprising:
  - a) PCR-amplifying a segment of MTRR nucleic acid using primers MSG108S (SEQ ID NO: 49) and AD292 (SEQ ID NO: 50), and
  - b) digesting the product of the PCR amplification reaction with the restriction enzyme *Nde* I, wherein a PCR product that is digested by *Nde* I indicates an increased risk of developing a neural tube defect in a mammalian embryo or fetus.
12. (Withdrawn) The method of claim 6, wherein said polymorphic MTRR is detected by analyzing MTRR polypeptide from said test subject.
13. (Original) The method of claim 6, wherein said test subject is a future female parent of said embryo or said fetus.
14. (Original) The method of claim 6, wherein said test subject is said embryo or said fetus.
15. (Withdrawn) The method of claim 6, said method further comprising detecting the presence of a polymorphic methylenetetrahydrofolate reductase (MTHFR) in a test subject, wherein detection of said polymorphic MTHFR indicates an increased risk of developing said neural tube defect, Down's Syndrome, or cardiovascular disease in said embryo or said fetus.
16. (Withdrawn) The method of claim 15, wherein said polymorphic MTHFR has a T instead of a C at a nucleotide position equivalent to position 677 of SEQ ID NO: 51.

17. (Withdrawn) The method of claim 15, wherein said polymorphic MTHFR is detected by analyzing nucleic acid from said test subject.

18. (Withdrawn) The method of claim 15, wherein said polymorphic MTHFR is detected by analyzing polypeptide from said test subject.

19. (Withdrawn) The method of claim 6, said method further comprising measuring the level of cobalamin in said test subject, wherein a low cobalamin level indicates an increased risk of developing said neural tube, cardiovascular disease or Down's Syndrome defect in said embryo or said fetus.

20. (Withdrawn) The method of claim 6, wherein said polymorphic MTRR contains a methionine instead of an isoleucine at amino acid position 22.

21. (Original) The method of claim 6, wherein said cardiovascular disease is premature coronary artery disease.

22-34 (Cancelled)

35. (New) A method for detecting an increased risk of Down's Syndrome, hyperhomocysteinemia, cardiovascular disease, or cancer in a mammal, said method comprising detecting the presence of a homozygous MTRR polymorphism that indicates an increased risk of Down's Syndrome, hyperhomocysteinemia, cardiovascular disease, or cancer in said mammal, wherein said polymorphism comprises

(a) a G instead of an A at position 66 relative to the first nucleotide of the start codon of MTRR,

(b) a G instead of an A at position 110 relative to the first nucleotide of the start codon of MTRR,

(c) a deletion of 4 nucleotides starting from position 1675 (nucleotides 1675-1678) relative to the first nucleotide of the start codon of MTRR, or

(d) a deletion of 3 nucleotides starting from nucleotide 1726 (nucleotides 1726-1728) relative to the first nucleotide of the start codon of MTRR.

36. (New) The method of claim 6, wherein said test subject is human.

37. (New) The method of claim 35, wherein said mammal is human.

38. (New) The method of claim 6, further comprising measuring the level of cobalamin in said test subject.

39. (New) The method of claim 35, further comprising measuring the level of cobalamin in said mammal.

40. (New) The method of claim 6, wherein said polymorphism comprises a G instead of an A at position 66 of MTRR.

41. (New) The method of claim 35, wherein said polymorphism comprises a G instead of an A at position 66 of MTRR.

42. (New) The method of claim 35, wherein said polymorphic MTRR is detected by analyzing nucleic acid from said test subject.

43. (New) The method of claim 35, wherein said cardiovascular disease is premature coronary artery disease.

## REMARKS

As presently claimed, the invention features methods for detecting an increased risk of developing neural tube defects, Down's syndrome, cardiovascular disease, hyperhomocysteinemia, or cancer in a mammal, or offspring derived therefrom, or in a mammalian embryo or fetus.

Examination of claims 1-3, 5-11, 13, 14, and 21-34 was reported in the Final Office Action dated November 22, 2002. All of these claims were finally rejected under 35 U.S.C. § 112, first paragraph. This rejection is addressed below.

### Support for the Amendments

For the record, Applicants disagree with the grounds for the present rejections. In the interest of expediting prosecution, claims 1-3, 5, 10, and 22-34 have been canceled. Applicants reserve the right to pursue these or related claims in a future application.

The limitation from canceled claim 10 has been incorporated into claim 1. Claim 1 has been amended to specify certain MTRR mutations, as disclosed, for example, on page 6, lines 5-15, of the specification. The diagnostic methods recited in new claims 35-37 and 39-43 are supported by, for example, page 6, lines 5-15; page 8, lines 14-20; page 13, line 21 through page 14, line 6; and page 25, lines 15-17. Support for new claim 38 is found, for example, on page 15, lines 6-10, and page 58. These amendments add no new matter.

### Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-3, 5-11, 13, 14, and 21-34 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner acknowledges that the specification is enabling for demonstrating the correlation between an increased risk of neural tube defects and a combination of the homozygous A66G methionine synthase reductase (MTRR) genotype and a low cobalamin level. The Examiner also acknowledges that the specification demonstrates a correlation between the A66G MTRR mutation and an increased risk for Down's Syndrome and coronary artery disease (CAD). Furthermore, the Examiner states that the specification is enabling for the association of homocysteine, folic acid, vitamin B6, and vitamin B12 with cancer and vascular

disease. However, the Examiner asserts that the specification fails to provide adequate guidance and evidence for the correlation between modulating MTRR biological activity and treating or preventing cancer, cardiovascular disease, neural tube defects, or Down's Syndrome in a subject. As noted above, treatment claims 1-3, 5, 10, and 22-34 have been canceled in the interest of expediting prosecution. Thus, this rejection with respect to these claims is now moot.

With respect to the remaining claims 6-9, 11, 13, 14, and 21, the Examiner states that the specification is not enabling for a method of detecting an increased risk of developing a neural tube defect, Down's Syndrome, or cardiovascular disease in any mammalian fetus or embryo that involves detecting any heterozygous or homozygous MTRR polymorphism in a parent, embryo, or fetus. Applicants respectfully traverse this aspect of the rejection.

Applicants have limited the pending claims to the detection of a G/A polymorphism at position 66 or 110 or a deletion of nucleotides 1675-1678 or 1726-1728 of MTRR. In particular, the claimed diagnostic methods involve the detection of either a homozygous MTRR polymorphism in a subject or the detection of a heterozygous or homozygous MTRR polymorphism in two future parents. Applicants further note that the present invention demonstrates that these polymorphisms in the human MTRR gene correlate with an increased risk of hyperhomocysteinemia and, in turn, with increased risk for cardiovascular disease, neural tube defects, cancer, and Down's Syndrome (see, for example, page 32, lines 22-24, and page 33, lines 1-4). For example, the specification teaches:

[s]evere deficiency of methionine synthase activity leads to megaloblastic anemia, developmental delay, hyperhomocysteinemia, and hypomethioninemia. Moreover, elevated plasma homocysteine is a risk factor in cardiovascular disease and neural tube defects (Rozen, *Clin. Invest. Med.* 19:171-178, 1996). (See, e.g., page 2, lines 15-18.)

Additionally, the specification states:

[w]e have cloned the gene encoding human methionine synthase reductase. This enzyme maintains methionine synthase in its reduced, activated state, and hence is an essential component of the methionine synthetic pathway. Deficiency of methionine synthase reductase results in hyperhomocysteinemia, a condition that has been implicated in cardiovascular disease and neural tube defects.

The presence of mutations in the methionine synthase reductase gene that decrease methionine synthase reductase enzymatic activity are likely to be associated with altered risk for cardiovascular disease, neural tube defects, and cancer. The invention features methods for risk detection and treatment of patients with hyperhomocysteinemia, cardiovascular disease, neural tube defects, and cancer. (See, e.g., page 4, line 19, through page 5, line 5.)

Furthermore, the specification teaches standard methods that can be used to rapidly identify a G/A polymorphism at position 66 or 110 or a deletion of nucleotides 1675-1678 or 1726-1728 in a MTRR nucleic acid of a subject (e.g., a parent of a future offspring, or a mammalian embryo or fetus, or other mammal), and thereby determine whether the subject, or a future offspring derived therefrom, has an increased risk for a neural tube defect, Down's Syndrome, hyperhomocysteinemia, cardiovascular, or cancer (see, for example, pages 8, 9, 49, 50, and 65 of the specification). For example, given Applicants' disclosure of primers to a mammalian MTRR nucleic acid (see for example, Table 1 and Figure 2), one skilled in the art of molecular biology could readily sequence mammalian MTRR nucleic acids to detect these polymorphisms.

In summary, the specification provides sufficient guidance and working examples so that no undue experimentation is required to diagnose an increased risk for a neural tube defect, hyperhomocysteinemia, cardiovascular disease, Down's Syndrome, or cancer in a subject, or a future offspring derived therefrom, based on the presence of a homozygous G/A polymorphism at position 66 or 110 or a homozygous deletion of nucleotides 1675-1678 or 1726-1728 of MTRR. In light of these teachings, Applicants submit that the specification fully enables the claims of the present invention. Therefore, the rejection of claims 1-3, 5-11, 13, 14, and 21-34 under 35 U.S.C. § 112, first paragraph, should be withdrawn.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that pending claims 6-9, 11, 13, 14, 21, and 35-43 are in condition for allowance, and a Notice of Allowance is respectfully solicited.

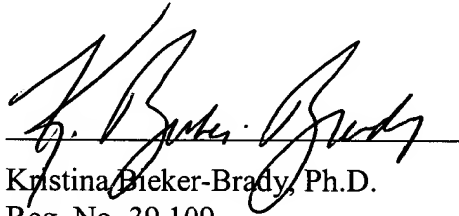
Enclosed is a petition to extend the period for replying for one month, to and including August 22, 2003, and a check for the required extension fee.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date:

August 15, 2003

  
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Kristina Dieker-Brady, Ph.D.  
Reg. No. 39,109

Clark & Elbing LLP  
101 Federal Street  
Boston, MA 02110  
Telephone: 617-428-0200  
Facsimile: 617-428-7045

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